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INTERACTION OF MICROCYSTIN-LR WITH SUPERCHAR: WATER DECONTAMINATION AND THERAPY

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ABSTRACT

Activated charcoal (SuperChar) has been recommended for therapeutic use against poisoning by several toxic agents, but it has not been tested against microcystin-LR toxicosis. Microcystin-LR, a cyclic heptapeptide isolated from fresh water blue-green algae, has been shown to be a potent hepatotoxin in animals and in man. Studies were performed to determine the degree of in vitro adsorption of microcystin-LR to SuperChar and to assess the efficacy of SuperChar as a therapeutic agent against microcystin-LR in vivo. Scatchard analysis of the in vitro data showed that microcystin-LR bound to SuperChar with a maximum binding capacity of 0.692 mM toxin/g SuperChar with a dissociation constant of 0.016 mM. The adsorption characteristics of microcystin-LR by SuperChar was applied successfully to the decontamination of water samples spiked with microcystin-LR. While an oral (po) dose of toxin mixed with SuperChar (0.31-0.36 g/kg) modulated the toxicity, an oral pretreatment with SuperChar did not prevent lethality induced by an oral or intraperitoneal (ip) dose of microcystin-LR in mice.

INTRODUCTION

Microcystins are a group of cyclic heptapeptides isolated from several strains of Microcystis aeruginosa (1). Microcystin-LR is a major toxic component of the freshwater (2,3) blooms of M. aeruginosa found worldwide (1,4,5). Toxins from these algae have been responsible for poisoning domestic and wild animals (6), and

present a potential health hazard to humans drinking from affected recreational water.

The unique properties of activated charcoal (nontoxic, large surface area, the ability to adsorb a wide variety of substances), have led to its use in the treatment of toxicosis from ingestion of toxic substances (7). SuperChar, a superactivated charcoal (3000 m^2/g), appears to bind greater quantities of material per unit weight than USP activated charcoal (8,9).

The purpose of this study was to measure the *in vitro* adsorption of microcystin-LR to SuperChar, and evaluate its usefulness for decontaminating water samples spiked with the toxin. In addition, we also investigated the effect of oral administration of SuperChar on the toxicity of microcystin-LR in mice.

MATERIALS AND METHODS

In Vitro Studies

Microcystin-LR, supplied by Dr. W. W. Carmichael of Wright State University, Dayton, OH, was approximately 95% pure as determined by high performance liquid chromatography (HPLC). In the adsorption studies, toxin stock solution (2 mg/ml) was prepared in 10% ethanol-water and diluted with water to obtain the appropriate concentrations. After dilution, the hepatotoxin-SuperChar suspension contained less than 1% ethanol.

SuperChar (Amoco AX-21, Anderson Development Co., Adrian, MI) in the commercially hydrated form (49%) was suspended (10 mg/ml, hydrated) in distilled water. Microcystin-LR (50-800 μg) was added to a vial containing a known amount of SuperChar suspension adjusted to 1.0 ml with distilled, deionized water. The samples were agitated at room temperature for 1 hr, then centrifuged at 1000 x g for 10 min in an Eppendorf centrifuge model 5414. Supernatants were analyzed for free microcystin-LR by HPLC and quantified by linear regression from a standard toxin curve (peak area vs toxin concentration).

Decontamination of Spiked Water Sample

A 13-mm Gelman disk filter (Nylon Acrodisc, 0.2 μm) was first packed with SuperChar (5 mg, hydrated) by passing through it 0.5 ml of 10 mg/ml SuperChar water suspension. A 20 μg sample of microcystin-LR in water (2 μg/ml) was passed through the Gelman-SuperChar disk filter at a rate of 0.2 ml/min, and 1-ml fractions were collected. Each fraction was analyzed for microcystin-LR by HPLC. A control sample of microcystin-LR (10 ml of 2 μg/ml solution) was passed through another disk filter with no SuperChar. There was no significant retention or adsorption of microcystin-LR to the membrane filter.

HPLC Analysis

Samples and standards were analyzed for microcystin-LR by HPLC (Beckman 450) with a manual injector (model 210A), pump (model 114M) and variable wavelength detector (model 165). Microcystin-LR was detected at 240 nm and quantified by measuring the peak area. Microcystin-LR was eluted on a C-18, 5-μm, 250 x 4.6 mm (BioRad) column maintained at 40° C. The mobile phase was 10 mM ammonium acetate:acetonitrile (74:26) at a flow rate of 1 ml/min.

Analysis of Adsorption Data

The data were analyzed by Scatchard plot (10) using a "dose effect analysis program" for microcomputers (Elsevier-Biosoft, Cambridge, CB2, 1LA, UK).

Animal Study

Male mice (CD-1, Charles River, Wilmington, MA), fed, weighing 28-32 g, were divided into groups (A through F), of six mice each. Group A received an oral (n=3) or an ip (n=3) dose of distilled water. Group B received only an ip dose of microcystin-LR (75 μg/kg). Group C received an oral dose of SuperChar (10 mg

hydrated weight, suspended in 0.5 ml water/mouse), followed 30 min later with an ip dose of microcystin-LR (75 µg/kg). Group D received an oral dose of microcystin-LR (5 mg/kg), while group E received an oral dose of SuperChar (10 mg hydrated weight, in 0.5 ml water/mouse) and then 30 min later, an oral dose of microcystin-LR (5 mg/kg). Group F received an oral dose of microcystin-LR (5 mg/kg) mixed with 10 mg (hydrated weight) SuperChar. Time to death and liver weights of each mouse were recorded. Animals that survived 24 hr after microcystin-LR challenge were killed and their liver weight recorded. Data were analyzed for statistical significance using t-distribution test for population means (11).

RESULTS

In Vitro Studies

The time required to achieve equilibrium (the same percent of microcystin-LR bound to SuperChar for two consecutive time periods) in microcystin-LR SuperChar binding was reached within 15 min. Therefore, for the remainder of the experiments, microcystin-LR was agitated with SuperChar at room temperature for 1 hr. Scatchard analysis of the data gave a maximum binding capacity (Bmax) and dissociation constant of 0.692 mM toxin/g SuperChar and 0.016 mM, respectively. The value of Bmax was used to calculate the amount of SuperChar applied to water decontamination and *in vivo* experiments.

The binding characteristics of microcystin-LR to SuperChar were applied successfully to the decontamination of water containing microcystin-LR (Table 1). In a small-scale experiment, 5 mg of SuperChar bound 98% of 20 µg of microcystin-LR.

Animal Studies

Microcystin-LR (75 µg/kg, ip or 5 mg/kg, po) caused a massive intrahepatic hemorrhage, reflected by an increase in the liver weight, and death in 100 percent of the mice within 1-2 hr (Table

TABLE 1

Decontamination by SuperChar of Water Sample Spiked With Microcystin-LR

Fraction No.	µg/Fraction A	B
1	0	0
2	0	0.04
3	0.021	0.022
4	0.033	0.028
5	0.166	0.086
6	0	0
7	0	0
8	0.03	0
9	0	0.044
10	0.051	0.09
Total µg eluted free	0.303	0.310
% Free	1.52	1.55
% Bound	98.48	98.45

Samples of 10 ml of microcystin-LR (2 µg/ml) were passed over a thin layer of SuperChar (5 mg,r=13mm) at a flow rate of 0.2 ml/min. One-ml fractions were collected and analyzed for free microcystin-LR, A and B are two separate experiments. % Free = (0.303/20) 100%.

2). The increase in liver weight after microcystin-LR administration was reported previously and confirmed to be due to hemorrhage (12). Administration of an oral dose of SuperChar (10 mg/mouse) did not alter the toxicity produced by an ip injection (Table 2, group C) or an oral dose (Table 2, group E) of microcystin-LR, reflected in the ratio of liver weight to body weight, mean time to death, and 24-hr mortality.

Mice that received an oral dose of microcystin-LR mixed with SuperChar (group F, Table 2), prior to its administration had 100% survival. These mice appeared normal by observing their movements, grooming, breathing, and feeding activities. Although liver weights were significantly lower than those intoxicated mice in group D (Table 2) they were, however, significantly higher than in the control group (group A, Table 2).

TABLE 2

Effect of Oral Dose of SuperChar on Microcystin-LR (MCY) Toxicity In Mice

Treatment	Group	MTD (min)	%liver	Mortality[a]
Water (po/ip)	A	b	4.17 ± 0.66	0/6
MCY (ip)	B	131.8 ± 30.0	7.59 ± 1.71	6/6
SuperChar/MCY (po/ip)	C	135.4 ± 31.3[c]	7.28 ± 0.76[c]	5/6
MCY (po)	D	61.2 ± 14.2	8.15 ± 0.38	6/6
SuperChar/MCY (po/po)	E	152.0 ± 90.2[c]	7.98 ± 0.35[c]	5/6
MCY + SuperChar (po)	F	b*	6.53*± 0.45	0/6*

a: mortality ratio for 24 hr post intoxication, dead/total.
b: all animals survived 24 hr.
c: mean ± SD of mice which died within 24 hr.
MTD: mean time to death (mean ± SD).
%liver: {liver weight (g)/body weight (g)}100, mean ± SD
A: control mice received water (n=3, ip; n=3, po).
B: MCY (75 μg/kg, ip).
C: SuperChar (10 mg/mouse, po) in 0.5 ml water, after 30 min, MCY (75 μg/kg, ip).
D: MCY (5 mg/kg, po).
E: SuperChar (10 mg/mouse, po) in 0.5 ml water, after 30 min, MCY (5 mg/kg, po).
F: SuperChar (10 mg/mouse) mixed with MCY (5 mg/kg), po in 0.5 ml water
* $p<0.05$ from group D and group A.

DISCUSSION

The calculated maximum binding capacity and dissociation constant for microcystin-LR adsorption to SuperChar indicates that microcystin-LR bound (0.692 mM toxin/g SuperChar) to SuperChar with a moderate binding affinity (0.016 mM). In comparison, pentobarbital was shown (9) to bind to SuperChar with a maximum binding capacity of 1.14 mmole/g SuperChar and an affinity constant

of 3.29 mM, while tilidine was shown (13) to bind activated charcoal with a maximum binding capacity of 185.5 mg/g of charcoal.

The interaction of microcystin-LR with SuperChar was exploited to achieve the decontamination of small volumes of water spiked with microcystin-LR. This application could be used to decontaminate larger volumes of water in areas where water supplies are contaminated by blooms of blue-green algae.

Since there was no difference in the lethality produced in mice administered an ip dose of microcystin-LR as compared to those receiving an oral dose of SuperChar followed by an ip dose of microcystin-LR, we conclude that SuperChar had no effect on the systemic clearance of microcystin-LR.

The in vitro interaction between SuperChar and microcystin-LR (mixed prior to po administration) was sufficient to abolish the lethal effects of the toxin in mice but not the hepatotoxicity. The observed hepatotoxicity (increased liver weight of group F vs. group A) may be related to desorption of sublethal quantities of toxin from SuperChar followed by absorption through the gut. Also, the interaction of SuperChar with microcystin-LR in the gut of the mouse may not be the same as it is in vitro.

Oral administration of activated charcoal is recommended in the treatment of overdose from agents such as atropine, phenytoin, theophylline, acetaminophen, carbamazepine, and amitriptyline (14-16). This recommendation is based upon the adsorption of these agents to charcoal in in vitro systems, as well as its effective therapeutic use in vivo. If treatment of microcystin-LR toxicosis is solely based upon its in vitro adsorption characteristics to SuperChar, a recommendation to use oral SuperChar in the treatment of toxicosis may be misleading. The in vivo study performed in mice indicates that administration of SuperChar in microcystin-LR intoxication is not an effective antidote. However, the application of SuperChar in water decontamination should be an effective means of eliminating the microcystin-LR from water supplies.

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